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(54) Title: VACCINE COMPOSITIONS COMPRISING CD–1 ANTIGENS AND T–CELL STIMULATING COMPOUND AND METHODS OF USE THEREOF

(57) Abstract

The present invention relates to immunogenic or vaccine compositions comprising a CD1 or lipid antigen and a T–cell stimulating compound (e.g., an adjuvant). The invention also pertains to methods for administering the immunogenic or vaccine compositions.

VACCINE COMPOSITIONS COMPRISING CD-1 ANTIGENS AND T-CELL STIMULATING COMPOUND AND METHODS OF USE THEREOF

RELATED APPLICATION(S)

This application claims the benefit of Provisional Application No. 60/081,638

5    filed on April 13, 1998, the entire teachings of which are incorporated herein by

reference.

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by a grant from the National

Institutes of Health. The Government has certain rights in the invention.

10   BACKGROUND OF THE INVENTION

Vaccination induces specific immunity in a host against foreign viruses,

bacteria or parasites. Many types of infectious agents and their products have been

used as vaccines. However, not all vaccine compositions are capable of eliciting an

immune response sufficient to protect the host against the challenge of infection.

15   Similarly, many diseases, disorders or conditions exist in which the immune

system is compromised or suppressed. Many treatments for such diseases or disorders

are incapable or ineffective to boost or stimulate the immune system. Examples of such

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diseases or conditions are the AIDS disease, or a cancer patient that has undergone chemotherapy.

These instances illustrate a need to develop a composition or drug that stimulates or enhances the immune response. In particular, a need exists to enhance the

5    immune response for vaccines that do not illicit a sufficient protective immune response in a host. Additionally, a need exists for a composition that stimulates the immune system of an individual whose immune system is compromised.

SUMMARY OF THE INVENTION

The present invention relates to methods of vaccinating or enhancing an immune

10    response in a mammal to at least one CD1 antigen that comprise administering (e.g., co-administering) to the mammal (e.g., individual) an effective amount of at least one CD1 antigen (e.g., an non-peptide or lipid antigen) and at least one T-cell stimulating compound (e.g., an adjuvant), wherein the CD1 antigen elicits a CD1-restricted response. The types of lipid antigens are described herein. Examples of adjuvants are

15    mineral salt adjuvants, Incomplete or Complete Freund's Adjuvants, Basille Calmette-Guerin adjuvants, block polymer adjuvants, cholera toxins, cytokines, CPG motif containing adjuvants, oil/water emulsion adjuvants, MF-59 adjuvants, LeIF adjuvants, liposome adjuvants, ISCOM adjuvants, Monophosphoryl A adjuvants, biodegradable microsphere adjuvants, muramyl dipeptide adjuvants, polyphosphozine adjuvants or

20    saponin adjuvants (e.g., QS-7, QS-17, QS-18 and QS-21). The present methods elicit at least one immunological parameter, such as an antibody response to the antigen, cytotoxic T-Lymphocyte response, T-cell proliferation response, helper T-cell response or a T-cell modulated cytokine response. The methods can be used to enhance or boost the immune response of an individual who has an immuno-compromised disease,

25    disorder or condition (e.g., AIDS or chemotherapy recipient). The present invention is used to elicit or boost an immune response for at least one bacterial infection (e.g., species of the *Mycobacteria* genus, *Haemophilus* genus, *Streptococcus* genus, *Staphylococcus* genus or *Chlamydia* genus) and /or at least one parasitic infection (e.g.,

species of the *Plasmodium* genus or *Trypanosoma* genus).  The CD1 antigen of the present invention can also be a tumor associated or derived antigen that is involved in diseases such as cancer (e.g., melanoma, breast cancer, prostate cancer and colo-rectal cancer) or a self antigen that is involved in autoimmune diseases (e.g., diabetes, Lupus,

5     rheumatoid arthritis).  An embodiment of the methods can further include administering a peptide-based antigen from the same organism or cancer type, so that an individual can obtain a protective or enhancing immunological effect against a non-peptide antigen as well as a peptide antigen.

        The invention also pertains to immunogenic or vaccine compositions that

10    comprise at least one T-cell stimulating compound (e.g., adjuvant), and at least one type of CD1 antigen (e.g., non-peptide or lipid antigen), wherein the CD1 antigen elicits a CD1-restricted response.  The CD1 molecules are a family of molecules including CD1a, CD1b, CD1c, CD1d and CD1e. Examples of adjuvants are described herein. As in the methods, the antigen of the composition is from at least one bacteria (e.g., species

15    of the *Mycobacteria* genus, *Haemophilus* genus, *Streptococcus* genus, *Staphylococcus* genus or *Chlamydia* genus) and/or at least one parasite infection (e.g., species of the *Plasmodium* genus or *Trypanosoma* genus).   Examples of the non-peptide antigen are a variety of lipids including a glycolipid, a phospholipid, a triglyceride, glycosylphosphatidylinositol (GPI), mycolic acid, glucose monomycolate (GMM),

20    lipoarabinomannan (LAM), lipo-oligosaccharide or phosphatidylinositolmannoside (PIM). The composition can optionally comprise a carrier.

        The present invention also include kits that comprise at least one T-cell stimulating compound (e.g., adjuvant), and at least one type of CD1 antigen (e.g., lipid antigen), wherein the CD1 antigen elicits a CD1-restricted response. The kit comprises

25    a CD1, e.g., lipid antigen from at least one bacteria and/or at least one parasite, and can optionally also comprise a peptide antigen (e.g., a peptide vaccine composition). Examples of adjuvants and types of lipid antigens are further described herein.

        The present invention provides more effective preventive and treatment options for individuals to obtain protection from serious bacterial and/or parasitic infections, or

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for cancer or autoimmune diseases, or immunotherapy for cancer or autoimmune

diseases, by administering an adjuvant/CD1 antigen composition.


BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic illustrating mycobacterial lipid antigen preparation from

5    *Mycobacterium tuberculosis.*

Figure 2A is a bar graph showing the response of lymphocytes in Counts Per

Minute (CPM), obtained from blood, to CD1-presented antigens: Ovalbumin (OVA),

*Mycobacterium phlei* glucose monomycolate (GMM), Organic Extract (O/E), Acetone

Fraction (Acetone Fx), Chloroform Fraction (CHC13 Fx), mycolic acid fractions (M.

10   Acid).

Figure 2B is a bar graph showing the response of lymphocytes in CPM, obtained

from the spleen, to CD1-presented antigens: Ovalbumin (OVA), *Mycobacterium phlei*

glucose monomycolate (GMM), Organic Extract (O/E), Acetone Fraction (Acetone Fx),

Chloroform Fraction (CHC13 Fx), mycolic acid fractions (M. Acid).

15   Figure 3A is a graph illustrating the *in vitro* lymphocyte response in CPM to a

purified lipid antigen, an acetone Fx ($\mu$g/mL), in immunized animals (guinea pigs)

combined with adjuvants Monophosphoryl Lipid A (MPL) and QS-21.

Figure 3B is a graph illustrating the *in vitro* lymphocyte response in CPM to

*Mycobacterium phlei* glucose monomycolate (GMM) ($\mu$g/mL) in immunized animal

20   (guinea pigs) combined with adjuvants Monophosphoryl Lipid A (MPL) and QS-21.

Figure 3C is a graph illustrating the *in vitro* lymphocyte response in CPM to

Ovalbumin (OVA) ($\mu$g/mL), in immunized animal (guinea pigs) combined with

adjuvants Monophosphoryl Lipid A (MPL) and QS-21.

Figure 4A is a graph showing the maximal proliferate response of CD4 depleted

25   cells (CPM) from an animal immunized with the following CD1 presented antigens

combined with adjuvants MPL and QS-21.: GMM, Acetone Fx, CHC13 Fx, M.acid,

and OVA.

Figure 4B is a bar graph showing the maximal proliferate response in CPM from an animal immunized with the following CD1 presented antigens combined with adjuvants MPL and QS-21.: GMM, Acetone Fx, CHC13 Fx, M.acid, and OVA.

Figure 5A is a graph showing the primary *ex vivo* cytotoxic T-lymphocyte responses (specific lysis (%)) against an ET ratio from animals immunized with Acetone fractions of either 50 mg/ml (Acetone 50) or 25 mg/ml (Acetone 25) combined with adjuvants MPL and QS-21 for a CD1b1 antigen presenting pathway.

Figure 5B is a graph showing the primary *ex vivo* cytotoxic T-lymphocyte responses (specific lysis (%)) against an ET ratio from animals immunized with Acetone fractions of either 50 mg/ml (Acetone 50) or 25 mg/ml (Acetone 25) combined with adjuvants MPL and QS-21 for a CD1c2 antigen presenting pathway.

Figure 5C is a graph showing the primary *ex vivo* cytotoxic T-lymphocyte responses (specific lysis (%)) against an ET ratio from animals immunized with Acetone fractions of either 50 mg/ml (Acetone 50) or 25 mg/ml (Acetone 25) combined with adjuvants MPL and QS-21 in a Mock or control system.

Figure 6 is a bar graph showing the proliferative response in CPM of CD1 restricted *Mycobacterium turbuculosis* lipid antigens specific T-cell lines (S2-CD8.1) raised from animals immunized with these lipid antigens.

Figure 7 is a bar graph illustrating the cytotoxic T-lymphocyte responses (specific lysis (%)) of specific T-cell line (S2-CD8.1) of *Mycobaterum turbuculosis* lipid antigen Organic Extract in 1, 10 and 20 μg/ml concentrations. This cell line lysed *M.turbuculosis* lipid antigen pulsed 104C1-CD1b1, CD1b2 and CD1c3 transfectants.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a vaccine composition or an immunogenic composition comprising at least one type of non-peptide antigen (e.g., a lipid antigen) and at least one T-cell stimulating compound (e.g., an adjuvant). The present invention utilizes the discovery that an Antigen Presenting Cell (APC) presents non-peptide antigens to T-cells in association with CD1 molecules.

A CD1 antigen such as a lipid or non-peptide antigen (e.g., from bacteria or parasites) is ingested by an APC (e.g., macrophage, B-cell or dentritic cell) and then represented in conjunction with a CD1 molecule, to thereby induce T-cell proliferation. (Porcelli, S.A. et al., "T-cell recognition of non-peptide antigens," *Current Opinion in*

5  *Immunology 8*:510-516 (1996)). This pathway is referred to herein as the "CD1 antigen-presenting pathway". The immune response that results from the CD1 antigen presenting pathway is referred to as a "CD1-restricted response." These non-peptide antigens are processed independently from the MHC peptide antigen-presenting pathway. U.S. Application No. 07/989,790 filed on December 10, 1992; U.S.

10  Application No. 08/080,072 filed on June 21, 1993; PCT Application No. PCT/US94/06991 filed on June 21, 1994; U.S. Patent No. 5,679,347 issued on October 21, 1997; U.S. Application No. 08/501,600 filed on July 12, 1995; U.S. Patent No. 5,853,737 issued on December 29, 1998; U.S. Application No. 08/501,491 filed on July 12, 1995; PCT Application No. PCT/US95/13274 filed on October 13, 1995; U.S.

15  Application No. 08/817,231 filed on April 11, 1997; U.S. Provisional Application No. 60/058,938 filed on September 12, 1997; U.S. Provisional Application No. 60/081,638 filed on April 13, 1998.

The present invention utilizes the concept that a CD1 antigen when combined with a T-cell stimulating compound, such as an adjuvant, elicits or boosts an immune

20  response to the non-peptide antigen. The composition that comprises a CD1 antigen and T-cell stimulating compound, produces a significantly more effective immunogenic response than the antigen alone. Hence, the invention relates to an immunogenic composition or vaccine composition comprising a CD1 antigen and a T-cell stimulating compound, as well as methods of use thereof.

25  The invention pertains to an immunogenic composition comprising at least one T-cell stimulating compound, and at least one CD1 antigen (e.g., the CD1 antigen/adjuvant or non-peptide/adjuvant composition), wherein the CD1 antigen elicits a CD1-restricted response. An immunogenic composition refers to a composition that contains these components and can produce or boost an immune response through the

CD1- antigen presenting pathway, as described herein. A CD1-restricted response is a response in which the CD1-antigen presenting pathway is utilized, and can be independent of the MHC antigen presenting pathway.

5    To "boost" the immune response refers to enhancing the immune response of an individual or mammal, as compared to the immune response prior to administration of the composition of the present invention. An increase or enhancement of various immunological parameters, as compared to a baseline (e.g., prior to administration of the composition) occurs. To "elicit" an immune response is to create an immune response to an antigen, sufficient to confer a protective or immune enhancing effect
10   against the CD1 antigen. In particular, T-cell proliferation occurs and/or antibodies are made in response to the antigen. Various immunological parameters can be measured to assess the enhancement or elicitation of an immune response, such as an antibody response to the antigen (e.g., level or titer), cytotoxic T-lymphocyte response, helper T-cell response, T-cell proliferative response, or a T-cell modulated cytokine response
15   (e.g., a cytokine that is modulated by the T-cell response). See Example 1. One or more of these immunological parameters increase in response to the CD1/adjuvant composition as compared to the responses prior to administration of the composition. One can measure a baseline level of the various immunological parameters prior to administering the compound and then reassess those levels after administering the
20   composition. An increase in one or more of the immunological parameters occurs, as compared to the baseline. The immunological parameters can also be measured against a control or levels from a control population to which the composition has already been administered (e.g., positive control) or to a population which has not been exposed to the composition (e.g., a negative control). These immunological parameters can be
25   measured utilizing methods known in the art. Accordingly, the present invention includes methods and vaccine compositions to elicit a protective effect against the CD1 antigen, as well as methods and immunogenic compositions for boosting or enhancing the immune response to the CD1 antigen.

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In addition to the methods and compositions that utilize the CD1 antigen/adjuvant composition, the present invention further includes eliciting an immune enhancing effect to peptide antigens. Several non-peptide antigens can be used to combat one or more diseases, and can be combined with peptide based vaccines to

5    produce a more effective and complete vaccine composition. The non-peptide antigen and adjuvant can be combined with one or more peptide antigens from the same organism or tumor cell to provide a composition which confers a protective or immune enhancing effect against a multitude of antigens, including peptide and non-peptide antigens. For example, a tuberculosis vaccine can include a *M. tuberculosis* encoded

10    peptide, a non-peptide antigen from the *M. tuberculosis* cell wall and an adjuvant. Such a vaccine can protect an individual from tuberculosis or disease caused by *M. tuberculosis*. Hence, the present invention includes vaccine or immunogenic compositions that comprise not only the non-peptide or lipid antigen and adjuvant, but also a peptide based antigen.

15    The administration of the CD1 antigen and the T-cell stimulating compound can occur simultaneously or sequentially in time. The T-cell stimulating compound can be administered before, after or at the same time as the CD1 antigen. Thus, the term "co-administration" is used herein to mean that the CD1 antigen and the T-cell stimulating compound will be administered at times to achieve the protective or immune enhancing

20    effect against the CD1 antigen. The methods of the present invention are not limited to the sequence in which the CD1 antigen and adjuvant are administered, so long as the adjuvant is administered close enough in time to produce the desired effect, e.g., boosting or eliciting a CD1 restricted response to the CD1 antigen. In a preferred embodiment, the administration of the non-peptide antigen/adjuvant composition occurs

25    simultaneously. The methods also include co-administration with other drugs that aid in eliciting or boosting an immunogenic response (e.g., cytokines such as IL-2, GM-CSF, and IL-12 and/or antibodies).

A CD1 antigen refers to an antigen that can undergo the CD1 antigen presenting pathway after its administration (e.g., an antigen that is presented to the immune system

in association with a CD1 molecule). A CD1 antigen is also referred to herein as "a

CD1-presented antigen," an antigen that can elicit or produce a CD1-restricted response.

A non-peptide antigen refers to an antigen in which the whole or a portion thereof is

not a peptide, e.g., does not contain two or more amino acids in which a carboxyl group

5      of one is united with an amino group of the other, with elimination of a water molecule.

A CD1 antigen has a hydrophobic moiety which binds nonspecifically to a CD1-

molecule, which in turn positions hydrophilic components of the antigen for highly

specific interactions with T-cell antigen receptors. A form of a CD1 antigen is a lipid

antigen.

10      A lipid antigen can be obtained from a variety of biological sources, or can be

synthesized. Such antigens can be isolated or synthesized by standard methods known

to those skilled in the art, so that characteristics which allow the non-peptide antigen to

be presented in the context of the CD1-antigen presenting pathway remain. These non-

peptide or lipid antigens can be isolated from a variety of biological sources including

15     bacteria (e.g., gram negative or gram positive), parasites, plants, fungus, or other

mammalian origins. The non-peptide antigen of the invention can be present in the

form of the whole, but inactivated bacteria or parasite; and/or bacterial or parasitic

portions thereof which retain their antigenicity. These lipid antigens are found in the

cell wall of bacteria, parasites and/or fungi. For example, lipid antigen components of

20     *Mycobacteria* can be isolated by standard methods described in Goren, M.B. and

Brennan, P.J., *Mycobacterial Lipids: Chemistry and Biologic Activities in Tuberculosis*,

1979, In *Tuberculosis*; ed. G.P. Youmans, pp. 63-193, (Philadelphia, WB Saunders

1979), and Hunter S.W., *et al.*, *J. Biol. Chem.* 261:12345-12531 (1986).

Lipid fractions can be prepared from cultures of bacteria (e.g., *Mycobacterium*

25     *tuberculosis*), as shown in Figure 1. Lipid fractions can be obtained using an alcohol

mixture such as acetone or methanol, or chloroform. Extraction with chloroform or

methanol separates the organic extract (O/E) from insoluble, dilapidated cell wall

fractions. The O/E is placed on a silicone column and eluded with chloroform, acetone,

and then methanol, which yields three purified fractions. The chloroform fraction

contains triglycerides and free fatty acids. The acetone fraction contains glycolipid, and the methanol fraction contains various phospholipids such as phosphatidyl inositol, phosphatidyl ethanolamine and phosphatidyl glycerol. Accordingly, a lipid fraction such as a chloroform, acetone, or methanol fraction can be used to isolate a lipid antigen

5  for the present invention. An "isolated" lipid antigenic fraction refers to a fraction containing a substantially purified amount of lipid molecules or antigens (e.g., greater than 80%, 85%, 90%, or 95%).

Examples of CD1 antigens include lipid antigens such as a lipoglycan, a glycolipid, a phospholipid, a triglyceride, glycosylphosphatidylinositol (GPI), mycolic

10  acid, glucose monomycolate (GMM), lipoarabinomannan (LAM), lipo-oligosaccharide or phosphatidylinositolmannoside (PIM). The lipid antigen can comprise lipid based molecules, other than those described herein.

Accordingly, the present invention includes immunogenic compositions or vaccine compositions that contain a portion of a organism (e.g., contains a CD1 or lipid

15  antigen), or the entire organism which has been altered to provide a protective or immune enhancing response without producing infection of the naturally occurring, live organism, e.g., without causing disease. Examples of these vaccines are live organism attenuative vaccines, killed organism vaccines, subunit vaccines, or toxoid vaccines. Examples of bacteria or parasites which contain non-peptide or lipid antigens that are

20  used in the present invention are bacteria or parasites such as species of the *Plasmodium* genus (malaria), *Mycobacteria* genus (tuberculosis and leprosy), *Streptococcus* genus (streptococcosis), *Staphylococcus* genus (staphylococosis and pneumonia), *Haemophilus* genus (influenza), *Chlamydia* genus (chlamydiosis), or the *Trypanosoma* genus (trypanosomiosis).

25       In particular, mycobacteria are a genus of aerobic intracellular bacterial organisms which upon invasion of their host, survive within endosomal compartments of monocytes and macrophages. Human Mycobacterial diseases include tuberculosis (caused by *M. tuberculosis*), leprosy (caused by *M. leprae*), Bairnsdale ulcers (caused by *M. ulcerans*) and various infections caused by *M. Marimum, M. kansasii, M.*

*scrofulaceum, M. szulgai, M. xenopi, M. fortuitum, M. chelonei, M. haemophilum and M. intracellulare.* Wolinsky, E. Chapter 37 in *Microbiology: Including Immunology and Molecular Genetics,* 3rd Ed., Harper & Row, Philadelphia 1980, Daniel. T.M., Miller, R.A. and Freedman, S.D., Chapters 119, 120, and 121, respectively, in

5    *Harrison's Principles of Internal Medicine,* 11th Ed. Braunwald. E., et al., eds., McGraw-Hill, New York, 1987.

With the advent of AIDS, tuberculosis is increasing at a nearly logarithmic rate, and multidrug resistant strains are appearing and now account for one third of all cases in New York City. Bloom, B.R. and Murry, C.J. L., *Science 257*:1055-1064 (1992).;

10   U.S. Congress, Office of Technology Assessment, *The Continuing Challenge of Tuberculosis,* OTA-H-574. U.S. Government Printing Office, Washington, D.C., 1993. Mycobacterial strains which were previously considered to be nonpathogenic strains (e.g., *M. avium*) have now become major killers of immunosuppressed AIDS patients. Moreover, current Mycobacterial vaccines are either inadequate, in the case of the BCG

15   vaccine to *M. th.,* or, with regard to *M. leprae,* unavailable. Kaugmann S., *Microbiol. Sci.* 4:324-328 (1987); U.S. Congress, Office of Technology Assessment. *The Continuing Challenge of Tuberculosis, pp*62-67. OTAH-574 U.S. Government Printing office, Washington, D.C., 1993. The present invention's methods and composition that boost the immune system is important in fighting such conditions.

20        The discovery of a structural motif for antigen presentation by CD1 proteins provides the means by which synthetic antigens can be used to extend the spectrum of antigens presented by CD1 molecules. The present invention encompasses vaccine compositions comprising non-peptide synthetically derived antigens that are effective against one or more gram negative, gram positive bacteria (including *Streptococcus* sp.

25   and/or *Staphylococcus* sp.), one or more parasites (including malaria) and one or more tumor antigens. All gram negative bacteria contain lipopolysaccharides (LPS) which are similar in structure to lipomannans. Most gram positive bacteria contain structurally-related glycolipids such as lipoteichoic acids. In addition, the chemical composition of

many disease-causing protozoa includes glycolipids such as the lipophosphoglycans of *Leishmania*. Orlandi, P.A. and S.J. Turco, *J. Biol. Chem.* 262:10384-10391.

The cell walls and other cellular components of the fungi also contain lipoglycans. Therefore, the present invention includes vaccine composition comprising a non-peptide/lipid synthetic antigen/adjuvant complex which can be used to prevent or treat fungal infections of mammals. These antigens can include partial derivatives of LAM, GMM, PIM molecules or derivatives of similar glycolipids from microbial organisms, whether prokaryotic or eukaryotic in nature.

The present invention uses various parasites or portions thereof as non-peptide antigens. Examples of diseases caused by protozoa include, but are not limited to, malaria, trichinosis, filariasis, trypanosomiasis, schistosomiasis, toxoplasmosis and leishmaniasis. Accordingly, the present invention provides methods and composition to treat parasite or protozoan infections.

The invention can utilize synthetic derived antigens that utilize the CD1-restricted pathway. A synthetic antigen, in accordance with this invention, is an antigen which is not naturally occurring in an organism. A synthetic antigen may be one that chemically synthesized, or parts thereof synthesized by combining elements, molecules or compounds. Further, a synthetic antigen can be constructed by combining two or more components, one or more of which is isolated or derived from an organism, thus producing a hybrid or chimeric antigen. A synthetic antigen, comprising a lipid moiety and a hydrophilic moiety which is chemically synthesized, can be presented to a T cell by an APC in association with CD1 molecules through the CD1 antigen presenting pathway.

The CD1 or lipid antigen is combined with a T-cell stimulating compound. A T-cell stimulating compound is a compound that can increase T-cell proliferation or T-cell helper effects when introduced to the immune system with an antigen. T-cell stimulating compounds include adjuvants. An adjuvant is a substance which enhances the immunogenic response to an antigen. In particular, adjuvants are generally considered to be in one of two categories: 1) "vehicles" that help to transport and retain

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antigens in lymphoid tissues, or 2) "immuno-modulates" that stimulate locally secreted cytokines. Adjuvants can composed of bacteria or bacterial products which contribute to the immunogenicity of the antigen. Examples of adjuvants are mineral salt adjuvants (alum or calcium based adjuvant), Incomplete or Complete Freund's Adjuvant, Basille

5    Calmette-Guerin (BCG) adjuvant, block polymer adjuvant (Titermax), cholera toxin (Cholera toxin-B-subunits, enterotoxin (LT) mutants), cytokines, CPG motif containing adjuvant, oil/water emulsion adjuvant (oil and water; water and oil; water, oil and water adjuvants), MF-59 adjuvants, LeIF adjuvants (e.g., protein based), liposome adjuvant, ISCOM adjuvant, Monophosphoryl A adjuvant (MPL), biodegradable microsphere

10   adjuvant, muramyl dipeptide adjuvant, polyphosphozine adjuvant, and saponin adjuvant (QS-7, QS-17, QS-18, or QS-21). Preferably, the compositions comprise two or more adjuvants (e.g., QS-21 and MPL).

Other lipid based vaccines are encompassed by the present invention. Individuals having diseases, such as cancer and autoimmune diseases, can be vaccinated

15   using a lipid/adjuvant composition. Cancers include melanoma, breast cancer, prostate cancer and colo-rectal cancer. Lipid based antigens from tumors can be used as the non-peptide antigen for the present invention. Similarly, autoimmune diseases such as diabetes, Lupus, and rheumatoid arthritis can be treated with non-peptide/lipid antigens.

A family of CD1 molecules exists that participates in the CD1-antigen

20   presenting pathway. In humans, the CD family is encoded by five non-polymorphic and closely linked genes located on a single chromosome. These show an intron/exon structure similar to that of MHC Class I genes and encode polypeptides with a modest but significant level of homology to both MHC Class I and II proteins. Five human CD1 proteins exist, and are CD1a, CD1b, CD1c, CD1d and CD1e. These CD1

25   molecules are involved in the CD1 antigen presenting pathway. Porcelli, S. et al., *Immun. Rev.*, 120:137-183 (1991).

The present invention can be used in mammals including humans, cats, dogs, horses, rodents, guinea pigs, etc. The present compositions and methods are intended for human as well as veterinarian use.

The composition of the present invention can be administered with or without a carrier. The terms "pharmaceutically acceptable carrier" or a "carrier" refer to any generally acceptable excipient or drug delivery composition that is relatively inert and non-toxic. Exemplary carriers include sterile water, salt solutions (such as Ringer's

5    solution), alcohols, gelatin, talc, viscous paraffin, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrolidone, calcium carbonate, carbohydrates such as lactose, sucrose, dextrose, mannose, albumin, starch, cellulose, silica gel, polyethylene glycol (PEG), dried skim milk, rice flour, magnesium stearate, and the like. Suitable formulations and additional carriers are described in Remington's

10   Pharmaceutical Sciences, (17th Ed., Mack Pub. Co., Easton, PA), the teachings of which are incorporated herein by reference in their entirety. Such preparations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like which do not deleteriously react with

15   the active compounds. They can also be combined where desired with other active substances, e.g., enzyme inhibitors, to reduce metabolic degradation. A carrier (e.g., a pharmaceutically acceptable carrier) is preferred, but not necessary to administer the compound.

The present invention provides a variety of pharmaceutical compositions. Such

20   compositions comprise a therapeutically (or prophylactically) effective amount of an immunogenic or vaccine compositions, including a CD1 antigen, adjuvant, peptide antigen and/or a carrier. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents, or preservatives. Typical preservatives can include, potassium sorbate, sodium metabisulfite, methyl paraben,

25   propyl paraben, thimerosal, etc.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The method of administration can dictate how the composition will be formulated. For example, the composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

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Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

The carrier can be added to the composition at any convenient time. In the case

5    of a lyophilized vaccine, the carrier can, for example, be added immediately prior to administration. Alternatively, the final product can be manufactured with the carrier.

The immunogenic vaccine compositions of the invention can be administered intravenously, parenterally, intramuscular, subcutaneously, orally, nasally, topically, by inhalation, by implant, by injection, or by suppository. The composition can be

10   administered in a single dose or in more than one dose (e.g., boosted) over a period of time to confer the desired effect.

For enteral or mucosal application (including via oral and nasal mucosa), particularly suitable are tablets, liquids, drops, suppositories or capsules. A syrup, elixir or the like can be used wherein a sweetened vehicle is employed. Topical application

15   can also be used for example, in intraocular administration. Alternative methods of administration can include an immune-stimulating complex (ISCOM) as described in U.S. patent No. 4,900,549 (or European Patent Publication No. 0 604 727 A1 (Publ. July 6, 1994). In addition to liposomes, viral vectors, microspheres, and microcapsules are available and can be used. See, Rabinovich, *supra*.

20   For diseases of the lungs, such as tuberculosis, pulmonary administration such as an inhaler may be preferred for prophylactic purposes or for immediate and specific localized treatment. Pulmonary administration can be accomplished, for example, using any of various delivery devices know in the art. See. *e.g.*, S.P. Newman (1984) in *Aerosols and the Lung*, Clarke and Davia (eds.), Butterworths, London, England, pp.

25   197-224; PCT Publication No. WO 92/16192; PCT Publication No. WO 91/08760; NTIS Patent.

For parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. In particular, carriers for parenteral administration include

aqueous solutions of dextrose, saline, pure water, ethanol, glycerol, propylene glycol, peanut oil, sesame oil, polyoxyethylene-polyoxypropylene block polymers, and the like. Ampules are convenient unit dosages.

5  Methods of administration will vary in accordance with the type of disorder and microorganism sought to be controlled or eradicated. The dosage of the vaccine will be dependent upon the amount of antigen, its level of antigenicity, and the route of administration. A person of ordinary skill in the art can easily and readily titrate the dosage for an immunogenic response for each antigen-adjuvant complex and method of administration.

10  The actual effective amounts of compound or drug can vary according to the specific composition being utilized, the mode of administration and the age, weight and condition of the patient, for example. As used herein, an effective amount of the drug is an amount of the drug which elicits or boosts an immune response to the non-peptide antigen. Dosages for a particular patient can be determined by one of ordinary skill in

15  the art using conventional considerations, (e.g. by means of an appropriate, conventional pharmacological protocol).

The present invention also relates to kits comprising at least one T-cell stimulating compound (e.g. adjuvant) and at least one non-peptide antigen (e.g., a lipid antigen) wherein the non-peptide antigen elicits a CD1 restricted response. As

20  described herein, the antigen can be obtained or isolated from at least one bacteria and/or parasite, or any other organism containing a lipid-based antigen. The antigen can also be a tumor associated (e.g., cancer) or a self (e.g., autoimmune disease) antigen. The kit can comprise various adjuvants as described herein. Preferably, the adjuvant is QS-7, QS-17, QS-18, or QS-21. Furthermore, the kit can comprise a peptide antigen or

25  vaccine, as described herein.

The following examples are meant to be illustrative and not limiting in any way.

EXEMPLIFICATION

Example 1: Measuring Immunological Parameters including Antibody Response, Cytokine Response and T-cell Response

Methods for assessing the antibody or cytokine response to a non-peptide antigen include several suitable assays that are known in the art. A sample from an
5    individual who has received the adjuvant/non-peptide antigen composition can be analyzed. The level of cytokines which are affected by T-cell proliferation can be measured, as well as the level of antibody specific to the non-peptide that is made. An increase in the level of cytokines and/or antibodies specific to the non-peptide antigen occurs after administration of the adjuvant/non-peptide antigen composition of the
10   present invention. The sample can be any biological component that contains the cytokine or antibodies to the antigen (e.g., blood sample, urine sample, sputum sample, etc.). Suitable assays encompass immunological methods, radioimmunoassay, Flow cytometric assays, enzyme-linked immunosorbent assays (ELISA) and chemiluminescence assays.
15   In determining the amounts of an antibody specific to the antigen to which the individual is being immunized, an assay includes combining or contacting the sample to be tested with the non-peptide antigen being assessed (e.g., the bacteria and/or parasitic antigen or portion thereof) under conditions suitable for formation of a complex between antibody and the antigen. The level of the complex formation is then detected
20   or measured (directly or indirectly).

To detect cytokine levels for cytokines that are influenced by T-cell proliferation, immunological assays can be used. Instead of contacting the sample with the non-peptide antigen, as is done for assessing the level of antibody in the sample, one can combine the sample containing the cytokine with an antibody specific to the
25   cytokine under conditions that are suitable for the formation of a complex between the cytokine and an anti-cytokine antibody. The level of the cytokine/anti-cytokine complex can then be detected.

The sample can be obtained directly or indirectly (e.g., provided by a healthcare provider), and can be prepared by a method suitable for the particular sample and the

assay format selected. For example, suitable methods for whole blood collection are venipuncture or obtaining blood from an in-dwelling arterial line. The container into which a healthcare provider deposits the blood can contain an anti-coagulant such as ACD-A, heparin, or EDTA.

5       Suitable labels can be detected directly, such as radioactive, fluorescent or chemiluminescent labels. They can also be indirectly detected using labels such as enzyme labels and other antigenic or specific binding partners like biotin. Examples of such labels include fluorescent labels such as fluorescein, rhodamine, CY5, chemiluminescent labels such as luciferase, radioisotope labels such as $^{32}P$, $^{125}I$, $^{131}I$,

10     enzyme labels such as horseradish peroxidase, and alkaline phosphatase, $\beta$-galactosidase, biotin, avidin, spin labels and the like. The detection of antibodies in a complex can also be done immunologically with a second antibody which is then detected (e.g., by means of a label). Conventional methods or other suitable methods can directly or indirectly label an antibody. The antibody or cytokine levels can be

15     assessed using known methods in the art, or those later developed.

The titer is determined by titration of the antiserum by serial dilution, and the point at which binding falls to 50% of the maximum. The titer is expressed as the reciprocal of the serum dilution which defines the end point.

T-cell responses can also be measured using methods known in the art. A

20     sample from an individual can be obtained, such as blood, bone marrow, lymphoid organs, epithelia and tissue or cells from an inflammation site. T-cell functions can be measured in various ways (e.g., target cell killing (CTL assays), macrophage activation, T-cell proliferation, B-cell activation, or lymphokine production.

T cells are detected by their effects on target cells displaying antigen, or the

25     secretion of specific cytokines that act on such target cells. Measuring these effector functions forms the basis for T-cell bioassays used to assess both T-cell specificity for antigen and T-cell effector functions.

Activated T cells (e.g., CD8 T-cells) generally kill any cells that display the specific peptide or lipid. Therefore, CD8 T-cell function can be determined using the

simplest and most rapid T-cell bioassay-the killing of a target cell by a cytotoxic T cell. This can be detected in a $^{51}$Cr-release assay. Live cells will take up, but do not spontaneously release, radioactively labeled sodium chromate, $Na_2^{51}CrO_4$. When these labeled cells are killed, the radioactive chromate is released and its presence in the

5     supernatant of mixtures of target cells and cytotoxic T cells can be measured.

In a similar assay, proliferating lymphocytes can be labeled with $^3$H-thymidine, which is incorporated into the replicating DNA. The cells are then collected and the incorporated CPM determined. These assays provide a rapid, sensitive, and specific measure of the activity of T cells.

10     CD4 T-cell functions involve the activation rather than the killing of cells bearing specific antigen. The activating effects of CD4 T cells on B cells or macrophages are mediated in large part by non-specific mediator proteins called cytokines, which are released by the T cell when it recognizes antigen. Thus, CD4 T-cell function is usually studied by measuring the type and amount of these released

15     proteins. As different effector T cells release different amounts and types of cytokines, one can learn about the effector potential of that T cell by measuring the proteins it produces. Cytokines can be detected by their activity in biological assays of cell growth, where they serve either as growth factors or growth inhibitors, or more specifically by a modification of ELISA, known as a capture or sandwich ELISA, as

20     described herein. In this assay, the cytokine is characterized by its ability to bridge between two monoclonal antibodies reacting with different epitopes on the cytokine molecule. Sandwich ELISA can also be carried out by placing the cells themselves on a surface coated with antibody to a cytokine. After a short incubation, the cytokine released by each cell is trapped on the antibody coat and the presence of cytokine-

25     secreting cells can be revealed when the cells are washed off and a labeled second anti-cytokine antibody is added. The cytokine released by each cell makes a distinct spot in this assay, which is therefore known as an ELISPOT assay. ELISPOT can also be used to detect specific antibody secretion by B cells, in this case using antigen-coated surfaces to trap specific antibody, and labeled anti-immunoglobulin to detect the bound

antibody. Sandwich ELISA avoids a major problem of cytokine bioassays, the ability of different cytokines to stimulate the same response in a bioassay. Bioassays must always be confirmed by inhibition of the response with monoclonal antibodies against the cytokine.

5 An alternative method is to identify cytokine mRNA, either in a cell population by reverse transcriptase-polymerase chain reaction (RT-PCR) or by *in situ* hybridization of single cells. Reverse transcriptase is an enzyme that is used by RNA viruses, like the human immunodeficiency virus that causes Acquired Immune Deficiency Syndrome (AIDS), to convert an RNA genome into a DNA copy, or cDNA. By harvesting mRNA

10 from T cells stimulated with antigen, one can make cDNA copies, which are then amplified selectively using cytokine-specific primers by the polymerase chain reaction. The amount of product is proportional to its representation in the RNA in the responding cell. *In situ* hybridization uses labeled anti-sense RNA probes to hybridize with sense RNA in single cells, either isolated from culture or directly in tissue sections.

15 This allows the number of cells making RNA encoding a particular cytokine to be determined. Janeway, Charles A., and Travers, Paul, "T-cell effector functions can be measured in four ways-target-cell killing, macrophage activation, B-cell activation, or lymphokine production," In *Immuno Biology, The Immune System in Health and Disease*, (New York and London: Garland Publishing Inc.), pp 2:31-2:33, the teaching

20 of which are incorporated by reference herein in their entirety.


Example 2: Methods for preparing lipid fractions, immunizing mammals, and other procedures for assessing immunological parameters.

 The lipid fractions disclosed herein (Acetone Fx, Methanol Fx, and Chloroform Fx) were prepared from cultures of *Mycobacterium tuberculosis* cells by standard

25 extraction methods. These extractions are diagramed in Figure 1. Following extraction with chloroform and methanol, the organic extract (O/E) was separated from the insoluble, delipidated cell wall fraction. The O/E was placed on a silica column and eluted with chloroform, acetone, and then methanol, which yielded three purified

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fractions. The chloroform fraction contained triglycerides and free fatty acids and represented 34% of the total extract by weight. A variety of glycolipids eluted with the acetone fraction which contained 12% of the extract. The methanol fraction comprised 29% of the extract and contained various phospholipids such as phosphatidyl inositol,

5   phosphatidyl ethanolamine and phosphatidyl glycerol. The delipidated cell walls were saponified and precipitated to yield isolated mycolic acid which represented 24% of the total cellular extract.

All fractions were tested for the presence of proteins by gel electrophoresis and standard protein assays, such as the Bradford assay and were found not to have

10  detectable protein. By quantitative amino acid analysis by acid hydrolysis and HPLC, the four fractions were determined to be relatively free of amino acids and all had less than a 5% amino acid content. In fact, the acetone and chloroform fractions each had less than 1% amino acid content.

Glucose monomycolate (GMM) was purified to homogeneity from rapidly-

15  growing *Mycobacterium phlei* cultures. *M. phlei* cells were hydrolyzed to yield GMM by drying on glass and treating with 2M TFA at 121° C for 2 hrs (G.S. Besra, *et al.* (1994) *Proc. Nat. Acad. Sci., USA 91*:12737). The yield of the resulting glycolipid was characterized by TLC in comparison with authentic GMM standards. ESI-MS analysis revealed ions of the expected m/z for GMM.

20  For immunization of Hartley strain guinea pigs, whole heat-killed *M. tuberculosis* cells or a mixture of the acetone, chloroform and methanol eluted fractions were combined with various adjuvants and, in some samples, incorporated into liposomes. Ovalbumin (OVA) was also added to mixtures. Ovalbumin stimulates immune responses of CD4$^+$ T cells through an MHC class II restricted response and

25  was a positive control demonstrating T cell activity to protein antigen.

Several adjuvants were combined with the lipid mixtures to enhance the immunogenicity of the antigen mixtures. Incomplete Freund's Adjuvant (IFA) is an oil-in-water emulsion. Titermax™ is a block copolymer (CRL89-41 or CRL-8300) combined with squalene and a microparticulate stabilizer (Vaxcel, Inc., Norcross,

Georgia, USA). QS-21 (Aquila Biopharmaceuticals, Inc., *supra*) and bone marrow

derived dendritic cells (BMDCs) were also used as adjuvants. Monophosphoryl Lipid

A (MPL) was purchased from RIBI ImmunoChem Research, Inc., Hamilton, MT

59840-3131.

5        Liposomes were produced from phospholipids which are known to be

nonstimulatory to the immune system of mammals. The ovalbumin and MPL

comprised a minor component of the liposomes which were used to deliver the lipid

mixture.

         Guinea pigs were inoculated with one of the above-described immunogenic

10      complexes. The administration was parenteral and subcutaneous. A second

administration was made 2-4 weeks apart. Cell samples were taken for *ex vivo*

measurements of T cell responses three weeks following the last immunization. The

results are shown in the Table *infra*.


         Example 3: Results showing a lipid antigen and adjuvant composition increases the

15      immune response in a mammal.

         Glycolipids mixed with QS-21, MPL or Bone Marrow Derived Dendritic Cells

(BMDCs) produced the best responses from T cells. QS-21 has been shown to boost

immune responses when combined with a wide variety of disease specific antigens.

Compared to the use of liposomes alone, the improved immunogenicity using an

20      adjuvant such as QS-21 is significant.

         Lymphocytes isolated from guinea pigs immunized with the isolated or purified

antigens in liposomes containing QS-21 and MPL responded to the lipid antigens *in*

*vitro*. Peripheral blood mononuclear cells responded to all antigens compared to

exposure to the cell medium alone. Medium devoid of antigen was used as a negative

25      control. See Figure 2A. The strongest responses were to OVA, GMM, and the organic

extract combining all the hydrophobic fractions. The peripheral blood mononuclear

cells were prepared by passing blood over a gradient to extract red blood cells and

granular monocytes. The eluate consisted of T and B cells and monocytes. This

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procedure, however, results in an enriched T cell population with less than 10% of immune-responding cells comprising B cells. Thus, the response demonstrated in Figure 2A is primarily a T cell response.

Splenic lymphocytes were purified by passing cells over a gradient and then
5   through nylon wool by standard procedures known to those of skill in the art to enrich the T cell population. These procedures result in a T cells to B cells ratio of greater than 10:1. Irradiated spleen cells are added in a ratio of 1:1 to include CD1$^+$ antigen presenting cells. These splenic fractions showed proliferation in the presence of OVA, GMM, organic extract, acetone and mycolic acid fractions. See Figure 2B.

10   When the cell suspension was treated with anti-CD4 antibodies to remove the CD4$^+$ T cell fraction, the response to OVA was diminished as shown in Figures 3A-3B and 4. The T cell fraction responding to the presence of antigens in these suspensions is comprised of CD4$^-$CD8$^-$ T cells and CD8$^+$ T cells. A comparison of these responses showing the significant responses to GMM, the acetone fraction, and mycolic acid is
15   shown in Figure 4. The antigen-specific response was dose-dependent and the highest values are compared.

This data presents the type of immunogenic complex capable of stimulating an immune response which will protect an animal from disease. Any CD1-presented antigen can be used in the immunogenic complexes of the invention. Examples of
20   structural requirements of a CD1-presented antigen can be found in Moody, D.B., *et al.*, *Science*, 278:283-286 (1997). Synthetic CD1-presented antigens can also be found therein. Of the adjuvants used, the best responses were observed with QS-21 and MPL or BMDCs. See Table 1.

SUMMARY OF EX VIVO CELL RESPONSES FROM IMMUNIZED HARTLEY STRAIN GUINEA PIGS

| | | | | T CELL PROLIFERATIVE RESPONSE TO: | | |
|---|---|---|---|---|---|---|
| ANIMAL | ANTIGEN | ADJUVANT | BOOST | ACETONE FX (Glycolipids) | CHLORO FX (Neutral Lipids) | METH FX (Phospholipids) |
| A021 | Killed M.Tb | IFA | Lipid/IFA x2 | ++ | + | - |
| A008 | Killed M.Tb | IFA | Lipid/IFA x2 | ++ | ++ | - |
| A009 | Lipid Mix | IFA | Lipid/FA x2 | - | - | - |
| A013 | Lipid Mix | Titermax | - | - | - | - |
| A014 | Lipid Mix | Titermax | - | - | - | - |
| A025 | Lipid Mix | Liposomes | - | - | - | - |
| A026 | Lipid Mix | Liposomes | Lipid/lipo x1 | + | + | +/- |
| A023 | Lipid Mix | BMDC's | Lipid/BMDCs x1 | ++ | + | ++ |
| A029 | Lipid Mix | Lipo/QS-21 | - | + | +/- | + |
| A031 | Lipid Mix | Lipo/QS-21 | Lipo/QS-21 x2 | + | + | - |
| A032 | Lipid Mix | Lipo/MPL | - | ++ | +/- | + |
| A034 | Lipid Mix | Lipo/MPL | Lipo/MPL x2 | + | + | - |

Lipid = mixture of Acetone (glycolipid), Chloroform (neutral lipid) and Methanol (phospholipid) eluted fractions.
BMDC=Bone marrow derived dendritic cells
Semiquantitative index of responses: +, stimulation 3-5 times greater than background proliferation in absence of antigen; ++, simulation >5 times background level; -, stimulation <3 times background level.

Example 4:     Results of Figures 5-7

        Figures 5 A-C show primary ex vivo cytotoxic responses of T lymphocytes

isolated from Strain 2 guinea pigs immunized with purified mycobacterial lipid antigens

reconstituted as an immunogenic composition by incorporation into liposomes with

5      adjuvants. Strain 2 guinea pigs were immunized with a purified lipid antigen mixture

that included M. tuberculsosis glycolipids (i.e., the lipid fraction eluted from a silica

colum using acetone as the solvent; refer to Figure 1 for details) inserted into liposomes

composed of inert phospholipids and cholesterol and mixed with the adjuvants QS-21

and MPL in the form of liposome twice. After immunization, the guinea pigs were

10     sacrificed and the splenic T-cells were obtained. The splenic T cells were cultured with

the M. tuberculosis glycolipid fraction for 6 days in the presence of irradiated splenic

adherent cells (i.e., a source of CD1+ antigen presenting cells) and 0.87 nM human

recombinant interleukin-2. Live cells were isolated and used as effector cells in a

standard chromium release assay for cytotoxic T cell activity. These T cells lysed target

15     cells that were engineered to express either of two different guinea pig CD1 molecules

(gpCD1b1 or gpCD1c2) by transfection of cDNAs encoding these molecules into a

guinea pig cell line (104C1) that normally lacks expression of CD1 molecules. Target

cell lysis was observed if the target cells were first incubated with M. tuberculosis

glycolipid antigens at a concentration of 50 ug/ml (i.e., represented as "Acetone 50" in

20     Fig. 5), and to a lesser extent if the target cells were incubated with the glycolipid

antigens at a concentration of 25 ug/ml (represented as "Acetone 25 in Fig. 5). No

cytotoxic activity of the T cells against any target cell was observed if the targets were

cultured in medium that did not contain mycobacterial lipid antigen (indicated as

"medium" in Fig. 5). This demonstrated that the cytotoxic T cells present in the culture

25     were lipid antigen specific, and that the antigen recognition was dose dependent. In

addition, no target cell lysis was observed if the target cells did not express CD1

molecules, which is the case for the 104C1 mock transfected targets (Figure 5C). This

demonstrated that the lipid antigen specific T cells present in the cultures were restricted

by either CD1b1 or CD1c2 molecules. Thus, these data show that both the CD1b1 and CD1c2 molecules are utilized and effective in this pathway to produce specific cytolytic T cells from an animal immunized using an appropriate immunogenic lipid antigen/adjuvant composition.

5          Figure 6 shows the proliferative response of a CD1-restricted M. tuberculosis lipid antigen specific T cells lines (S2-CD8.1) raised from the animal immunized with CD1-presented antigens. A strain 2 guinea pig was immunized twice by the subcutaneous route using an immunogenic lipid antigen/adjuvant formulation The proliferative responses (Counts Per Minute (CPM)of tritiated thymidine incorporation)

10   of the T cell line were measured in the presence of the T cell line alone; the T cell line cultured together with bone marrow derived dendritic cells (BM-DCs; a source of CD1+ antigen presenting cells); the T cell line plus BM-DCs plus a monoclonal antibody specific for all currently known forms of guinea pig CD1 (anti-CD1mAb); the T cell line plus BM-DCs and the M. tuberculosis lipid antigens (a total lipid

15   chloroform/methanol extract of M. tuberculosis, indicated as "M.Tbc O/E"; consult Figure 1 for details); and the T cell line plus BM-DCs and the M. tuberculosis lipid antigens and anti-CD1mAb. The results show that the T cells proliferated strongly when exposed to lipid antigens in the presence of CD1+ antigen presenting cells (BM-DCs), but not in the absence of CD1+ antigen presenting cells. This proliferation was

20   dependent on the presence of the lipid antigens, and was strongly inhibited by the anti-CD1 mAb. These data indicate that T cell lines that are both specific for lipid antigens and restricted by CD1 molecules can be derived from guinea pigs immunized using an appropriate immunogenic lipid antigen/adjuvant composition.

          Figure 7 illustrates the CD1 restriction elements of M. tuberculosis lipid antigen

25   specific T cell line (S2-CD8.1). S2-CD8.1 T cell line lysed M. tuberculosis lipid antigen pulsed 104C1/CD1b1.-b2 and c3 transfectants, but not a mock transfectant or transfectants expressing CD1b3, -b5 or -c2. T cell line S2-CD8.1 was derived from a strain 2 guinea pig immunized with an immunogenic lipid antigen/adjuvant composition consisting of the M. tuberculosis total lipid extract inserted into liposomes composed of

inert phospholipids and cholesterol and mixed with the adjuvants QS-21 and MPL. After immunization, the guinea pigs were sacrificed and T cells were isolated from the spleen. These T cells were stimulated four times in vitro by culture with a CD1+ antigen presenting cell (BM-DCs) plus M. tuberculosis lipid extract at 20 ug/ml. The

5    resulting T cell lines were expanded by the addition of human recombinant interleukin-2, and the cells were harvested and used in a standard chromium release assay to assess the presence of lipid antigen specific cytotoxic T cells and their restriction by individual members of the CD1 family. The results demonstrated significant lipid antigen dependent lysis of 104C1 target cells that had been transfected to express either CD1b1,

10   -b2 or -c3 molecules. This recognition was dose dependent, showing generally greater responses when the target cells were incubated with lipid antigen at 20 ug/ml compared to 10 ug/ml. In contrast, no significant lysis was observed of mock transfected 104C1 cells that do not express CD1 molecules in the presence or absence of lipid antigens. Furthermore, three other forms of guinea pig CD1 (CD1b3, b5 and -c2) proved to be

15   minimally active or inactive at endowing the 104C1 cells with the ability to present the lipid antigens to T cell line S2-CD8.1. Thus, these data show that the CD1 antigen presenting pathway is effective in producing an immune response for mammals that are immunized with an appropriate immunogenic lipid antigen/adjuvant composition.


The relevant teachings of all the references, patents and/or patent applications

20   cited herein are incorporated herein by reference in their entirety.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

## CLAIMS

What is claimed is:

1.    A method of enhancing an immune response in a mammal to at least one CD1 antigen comprising co-administering to the mammal an effective amount of at least one CD1 antigen and at least one T-cell stimulating compound.

2.    The method of claim 1, wherein the T-cell stimulating compound is an adjuvant.

5    3.    The method of Claim 2, wherein the adjuvant is selected from the group consisting of: mineral salt adjuvant, Incomplete or Complete Freund's Adjuvant, Basille Calmette-Guerin adjuvant, block polymer adjuvant, cholera toxin, cytokines, CPG motif containing adjuvant, oil/water emulsion adjuvant, MF-59 adjuvants, LeIF adjuvants, liposome adjuvant, ISCOM adjuvant,

10    Monophosphoryl A adjuvant, biodegradable microsphere adjuvant, muramyl dipeptide adjuvant, polyphosphozine adjuvant, and saponin adjuvant.

4.    The method of claim 3, wherein saponin adjuvant is selected from the group consisting of: QS-7, QS-17, QS-18 and QS-21.

5.    The method of claim 2, wherein the CD1 antigen is a lipid antigen.

15    6.    The method of claim 5, wherein at least one immunological parameter is elicited.

7.     The method of claim 6, wherein the immunological parameter is selected from the group consisting of: an antibody response to the antigen, cytotoxic T-Lymphocyte response, helper T-cell response, proliferative T-cell response, and a T-cell modulated cytokine response.

5     8.     The method of claim 7, wherein the mammal has an immuno-compromised disease, disorder or condition.

9.     The method of claim 8, wherein the composition is used to elicit or boost an immune response for at least one bacterial infection and /or at least one parasitic infection.

10     10.     The method of claim 9, wherein the composition is used to elicit or boost an immune response against malaria, leprosy, tuberculosis, streptococcosis, staphylococosis, pneumonia, influenza, chlamydiosis or trypanosomiosis.

11.     A method of vaccinating a mammal against at least one CD1 antigen, comprising administering to the mammal an effective amount of at least one
15     CD1 antigen and at least one adjuvant.

12.     The method of claim 11, wherein at least one immunological parameter is elicited.

13.     The method of claim 12, wherein the immunological parameter is selected from the group consisting of: an antibody response to the antigen, cytotoxic T-
20     Lymphocyte response, helper T-cell response, proliferative T-cell response and a T-cell modulated cytokine response.

14.   The method of claim 13, wherein the CD1 antigen is selected from the group

      consisting of: a glycolipid, a phospholipid, a triglyceride,

      glycosylphosphatidylinositol, mycolic acid, glucose monomycolate,

      lipoarabinomannan, lipo-oligosaccharide, phosphatidylinositolmannoside, a self-

5     antigen and a tumor derived antigen.


15.   The method of claim 14, wherein the response is elicited against at least one

      bacterial infection and/or at least one parasitic infection.


16.   The method of Claim 15, wherein the composition is used to elicit an immune

      response against malaria, leprosy, tuberculosis, streptococcosis, staphylococosis,

10    pneumonia, influenza, chlamydiosis or trypanosomiosis.


17.   The method of Claim 16, wherein the adjuvant is selected from the group

      consisting of: mineral salt adjuvant, Incomplete or Complete Freund's

      Adjuvant, Basille Calmette-Guerin adjuvant, block polymer adjuvant, cholera

      toxin, cytokines, CPG motif containing adjuvant, oil/water emulsion adjuvant,

15    MF-59 adjuvants, LeIF adjuvants, liposome adjuvant, ISCOM adjuvant,

      Monophosphoryl A adjuvant, biodegradable microsphere adjuvant, muramyl

      dipeptide adjuvant, polyphosphozine adjuvant, and saponin adjuvant.


18.   The method of claim 17, wherein saponin adjuvant is selected from the group

      consisting of: QS-7, QS-17, QS-18 and QS-21.


20  19.   The method of claim 11, further comprising administering at least one other

          antigen comprising a peptide antigen.


20.   A method of stimulating a CD1-restricted immune response in a mammal

      comprising administering to the mammal an effective amount of a composition

that comprises at least one adjuvant and at least one lipid antigen, wherein the
lipid antigen elicits a CD1-restricted response.

21.    The method of claim 20, wherein the lipid antigen is selected from the group
       consisting of: a glycolipid, a phospholipid, a triglyceride,
       glycosylphosphatidylinositol, mycolic acid, glucose monomycolate,
       lipoarabinomannan, lipo-oligosaccharide and phosphatidylinositolmannoside.

22.    The method of claim 21, wherein the response is elicited against at least one
       bacterial infection and/or at least one parasitic infection.

23.    The method of Claim 22, wherein the composition is used to elicit an immune
       response against malaria, leprosy, tuberculosis, streptococcosis, staphylococosis,
       pneumonia, influenza, chlamydiosis or trypanosomiosis.

24.    The method of Claim 22, wherein the adjuvant is selected from the group
       consisting of: mineral salt adjuvant, Incomplete or Complete Freund's
       Adjuvant, Basille Calmette-Guerin adjuvant, block polymer adjuvant, cholera
       toxin, cytokines, CPG motif containing adjuvant, oil/water emulsion adjuvant,
       MF-59 adjuvants, LeIF adjuvants, liposome adjuvant, ISCOM adjuvant,
       Monophosphoryl A adjuvant, biodegradable microsphere adjuvant, muramyl
       dipeptide adjuvant, polyphosphozine adjuvant, and saponin adjuvant.

25.    The method of claim 24, wherein saponin adjuvant is selected from the group
       consisting of: QS-7, QS-17, QS-18 and QS-21.

26.    An immunogenic composition comprising:
       a.    at least one T-cell stimulating compound, and

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b.    at least one CD1 antigen, wherein the CD1 antigen elicits a CD1-restricted response.

27.    The composition of claim 26, wherein the T-cell stimulating compound is an adjuvant.

28.    The composition of claim 27, wherein the CD1 antigen is a lipid antigen.

29.    The composition of Claim 28, wherein the antigen is from at least one bacteria and/or at least one parasite.

30.    The composition of Claim 29, wherein the bacteria is from a species selected from the group consisting of: *Mycobacteria* genus, *Haemophilus* genus, *Streptococcus* genus, *Staphylococcus* genus, and *Chlamydia* genus.

31.    The composition of Claim 30, wherein the parasite is from a species of the *Plasmodium* or *Trypanosoma* genus.

32.    The composition of Claim 31, wherein the adjuvant is selected from the group consisting of: mineral salt adjuvant, Incomplete or Complete Freund's Adjuvant, Basille Calmette-Guerin adjuvant, block polymer adjuvant, cholera toxin, cytokines, CPG motif containing adjuvant, oil/water emulsion adjuvant, MF-59 adjuvants, LeIF adjuvants, liposome adjuvant, ISCOM adjuvant, Monophosphoryl A adjuvant, biodegradable microsphere adjuvant, muramyl dipeptide adjuvant, polyphosphozine adjuvant, and saponin adjuvant.

33.    The composition of claim 32, wherein saponin adjuvant is selected from the group consisting of: QS-7, QS-17, QS-18 and QS-21.

34.     The composition of Claim 28, wherein the antigen is selected from the group
        consisting of a glycolipid, a phospholipid, a triglyceride,
        glycosylphosphatidylinositol, mycolic acid, glucose monomycolate,
        lipoarabinomannan, lipo-oligosaccharide, phosphatidylinositolmannoside, a self-
        antigen and a tumor derived antigen.

35.     The composition of Claim 27, wherein the antigen elicits a CD1 restricted
        response using a CD1 molecule selected from the group consisting of: CD1a,
        CD1b, CD1c, CD1d and CD1e.

36.     The composition of Claim 27, further comprising a carrier.

37.     A method for eliciting an immunogenic response in a mammal, comprising
        administering the composition of Claim 26.

38.     A vaccine composition comprising:
        a) at least one adjuvant, and
        b) at least one lipid antigen, wherein the lipid antigen elicits a CD1 restricted
        response.

39.     The vaccine composition of Claim 38, wherein the vaccine composition elicits
        an immune response against at least one bacterial infection and/or at least one
        parasitic infection.

40.     The vaccine composition of Claim 39, wherein the vaccine composition elicits
        an immune response against malaria, leprosy, tuberculosis, streptococcosis,
        staphylococosis, pneumonia, influenza, chlamydiosis or trypanosomiosis.
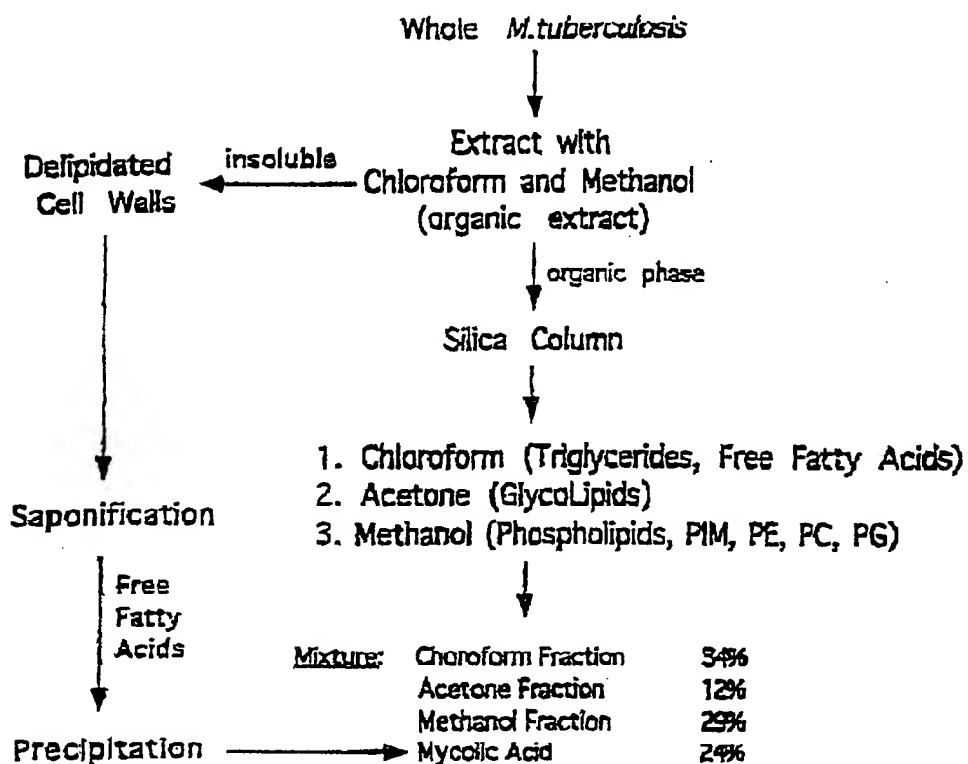
41. The vaccine composition of Claim 39, wherein the adjuvant is selected from the group consisting of: mineral salt adjuvant, Incomplete or Complete Freund's Adjuvant, Basille Calmette-Guerin adjuvant, block polymer adjuvant, cholera toxin, cytokines, CPG motif containing adjuvant, oil/water emulsion adjuvant,

5      MF-59 adjuvants, LeIF adjuvants, liposome adjuvant, ISCOM adjuvant, Monophosphoryl A adjuvant, biodegradable microsphere adjuvant, muramyl dipeptide adjuvant, polyphosphozine adjuvant, and saponin adjuvant.

42. The vaccine composition of claim 40, wherein saponin adjuvant is selected from the group consisting of: QS-7, QS-17, QS-18 and QS-21.

10   43. The vaccine composition of Claim 38, wherein the lipid antigen is selected from the group consisting of a glycolipid, a phospholipid, a triglyceride, glycosylphosphatidylinositol, mycolic acid, glucose monomycolate, lipoarabinomannan, lipo-oligosaccharide and phosphatidylinositolmannoside.

44. The vaccine composition of Claim 38, wherein the antigen elicits a CD1

15      restricted response using a CD1 molecule selected from the group consisting of: CD1a, CD1b, CD1c, CD1d and CD1e.

45. The vaccine composition of Claim 38, further comprising a carrier.

46. The vaccine composition of claim 39, further comprising a peptide antigen, wherein the peptide antigen elicits a non-CD1 restricted response.

20   47. A method of vaccinating a mammal, comprising administering the vaccine composition of Claim 38.

48. A kit comprising:

a) at least one T-cell stimulating compound, and

b) at least one CD1 antigen, wherein the CD1 antigen elicits a CD1-restricted response.

49.     The kit of claim 48, wherein the T-cell stimulating compound is an adjuvant.

5    50.     The kit of claim 49, wherein the CD1 antigen is a lipid antigen.

51.     The kit of Claim 50, wherein the antigen is from at least one bacteria and/or at least one parasite.

52.     The kit of Claim 51, wherein the adjuvant is selected from the group consisting of: mineral salt adjuvant, Incomplete or Complete Freund's Adjuvant, Basille
10    Calmette-Guerin adjuvant, block polymer adjuvant, cholera toxin, cytokines, CPG motif containing adjuvant, oil/water emulsion adjuvant, MF-59 adjuvants, LeIF adjuvants, liposome adjuvant, ISCOM adjuvant, Monophosphoryl A adjuvant, biodegradable microsphere adjuvant, muramyl dipeptide adjuvant, polyphosphozine adjuvant, and saponin adjuvant.

15   53.     The kit of claim 52, wherein saponin adjuvant is selected from the group consisting of: QS-7, QS-17, QS-18 and QS-21.

54.     The kit of claim 48, further comprising a carrier.

# Mycobacterial Lipid Antigen Preparation

## Direct purification from bacilli:

Whole  *M.tuberculosis*

↓

Extract with
Chloroform and Methanol
(organic extract)

Delipidated ← insoluble ──────

Cell Walls

↓ organic phase

Silica Column

↓

1. Chloroform (Triglycerides, Free Fatty Acids)
2. Acetone (GlycoLipids)
3. Methanol (Phospholipids, PIM, PE, PC, PG)

Saponification

↓ Free Fatty Acids

↓

| Mixture: | Chloroform Fraction | 34% |
|---|---|---|
| | Acetone Fraction | 12% |
| | Methanol Fraction | 29% |

Precipitation ──────→ Mycolic Acid   24%

Currently Available:        Approx. 1000 grams of M.Tb. (H37Ra) bacilli

7000 mg crude organic extract
350 mg chloroform eluted fx.
300 mg acetone eluted fx.
300 mg methanol eluted fx.
1500 mg mycolic acids
10 mg LAM

Fig. 1

Lymphocytes from guinea pigs immunized with purified lipid antigens. respond to lipid antigens _in vitro_



A050-1 Nylon wool-passed splenic T cells
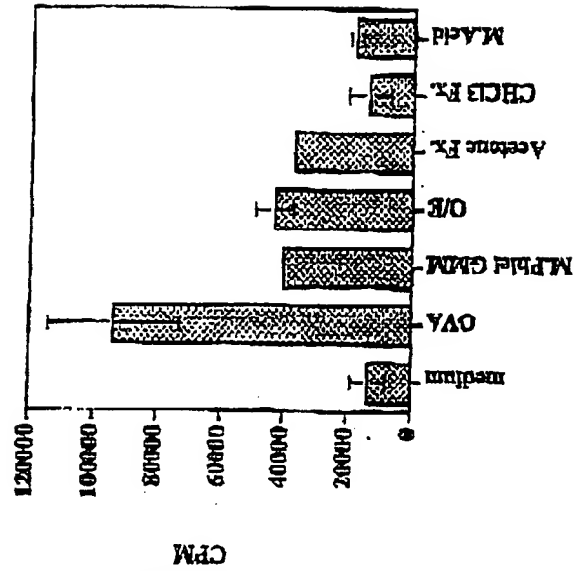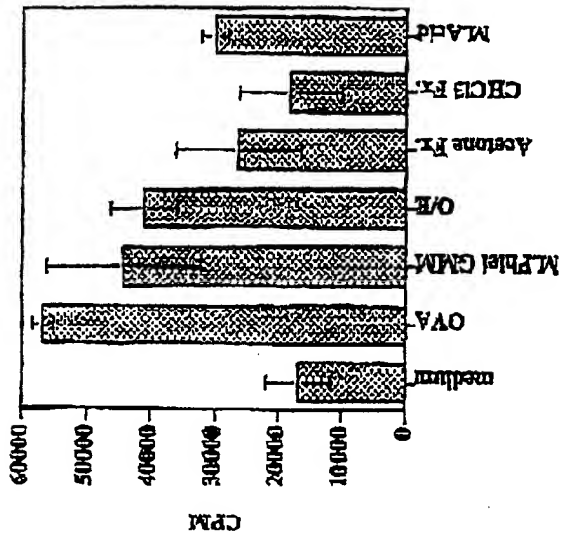
Fig. 2B

A050-1 PBMC

Fig. 2A

Responses to lipid antigens in immunized guinea pigs are present in the CD4 negative T cell fraction

A050-2
CD4-depleted PBMC

Hartley Guinea pig 6 wk after immunization
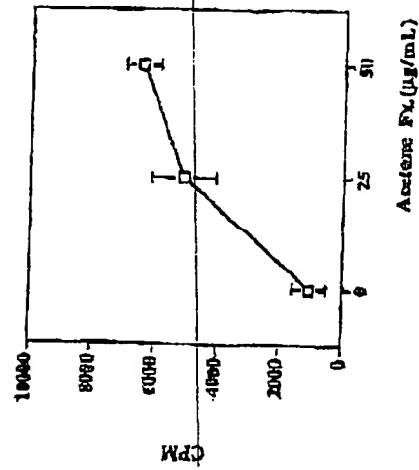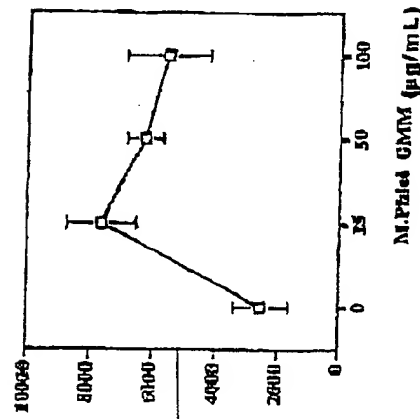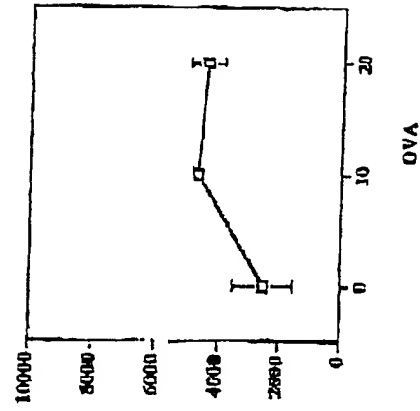Ag: Acetone Fx, CHCl3 Fx, M.acid, GMM/ liposome-MPL + QS 21+OVA

Fig. 3A

Fig. 3B

Fig. 3C

A050-2
CD4-depleted PBMC

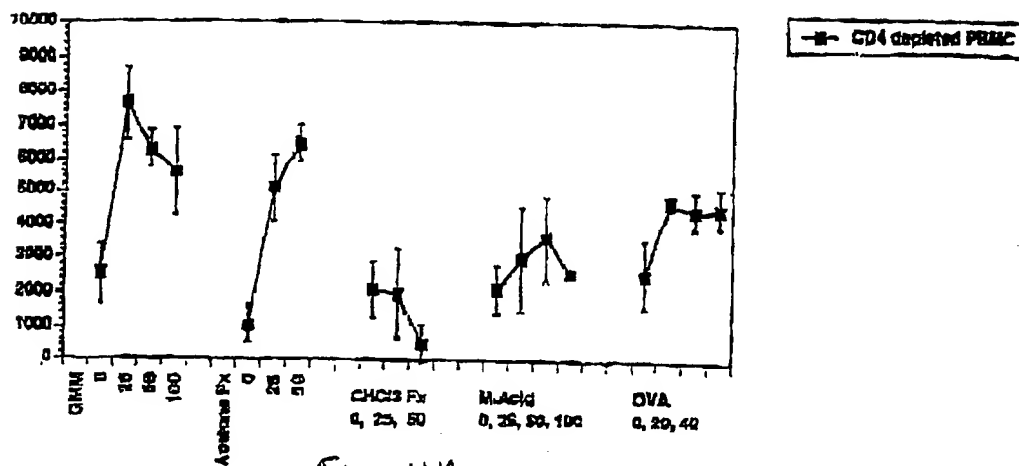Hartley Guinea pig 6 wk after immunization
Ag; Acetone Fx, CHCl3 Fx, M.acid, GMM/ liposome-MPL + GS 21+OVA
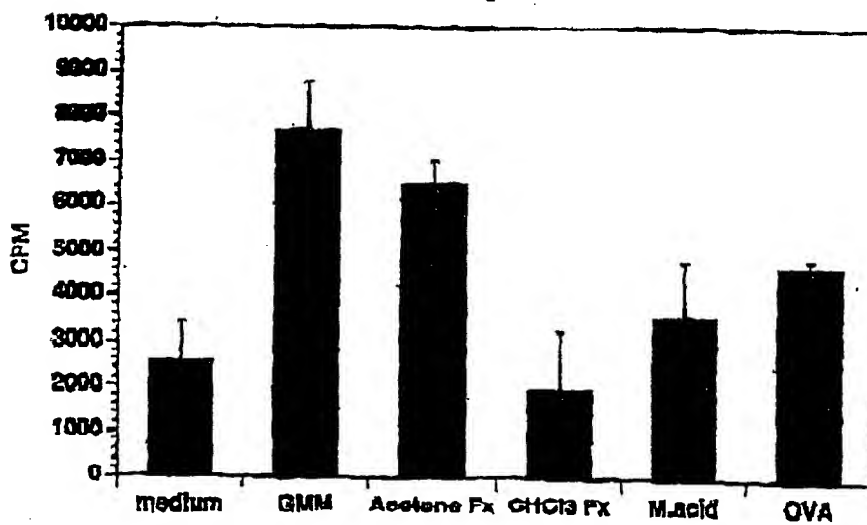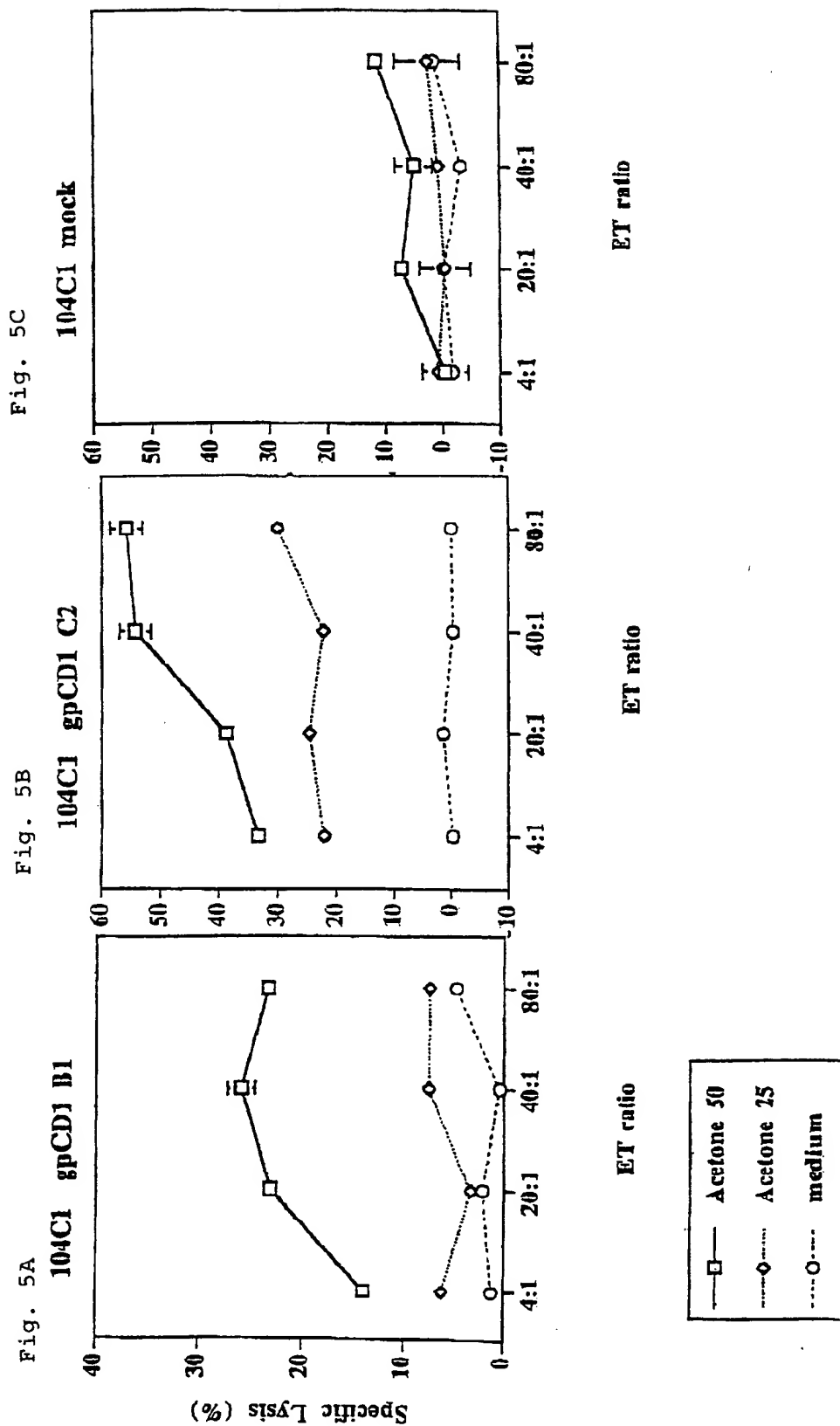


Fig. 4A



Fig. 4B

Fig. 5C

104C1 mock

Fig. 5B

104C1 gpCD1 C2

Fig. 5A

104C1 gpCD1 B1

Acetone 50
Acetone 25
medium

Proliferative responses of CD1 restricted M.Tbc lipid Ag-specific T cell line (S2-CD8.1)



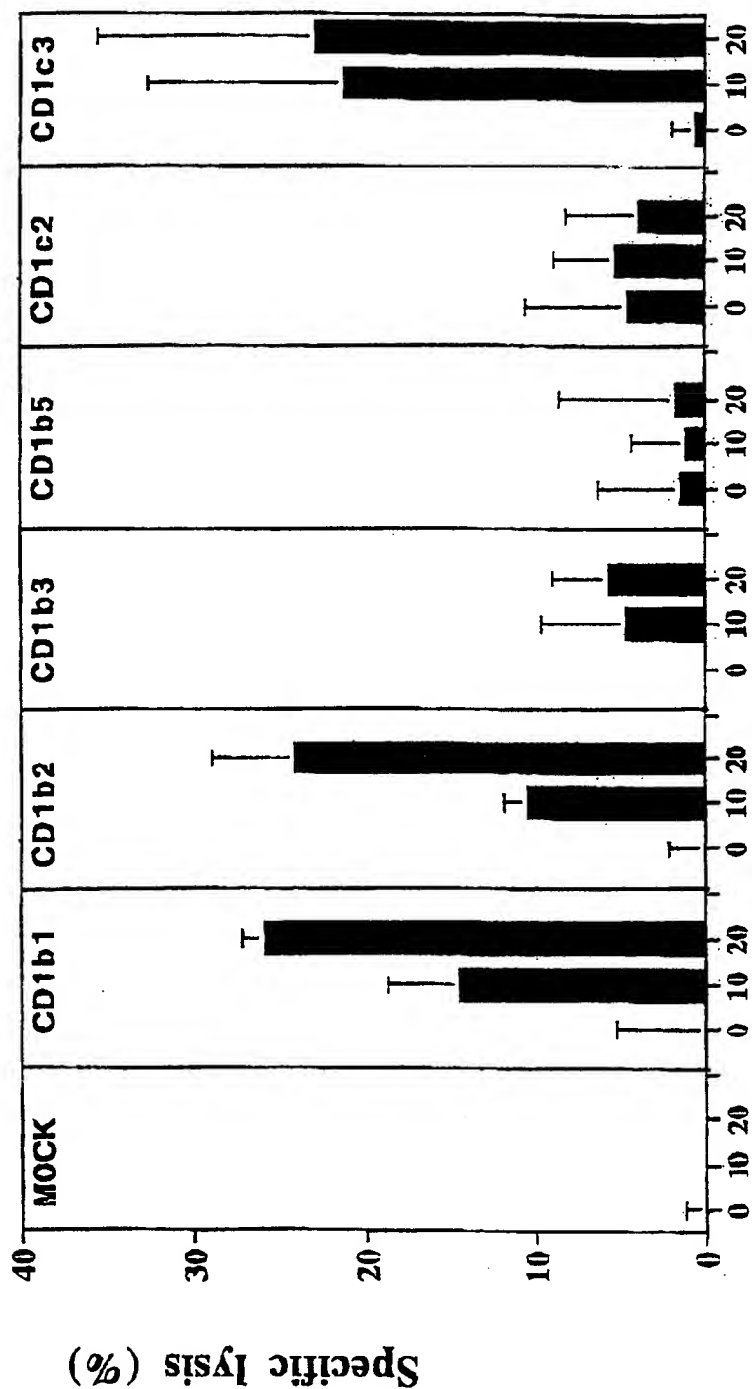| | | | | | |
|---|---|---|---|---|---|
| T cell line | + | + | + | + | + |
| CD1+ BM-DCs | - | + | + | + | + |
| M.Tbc O/E | - | - | - | + | + |
| anti-CD1mAb (CD1F2/6B5) | - | - | + | - | + |
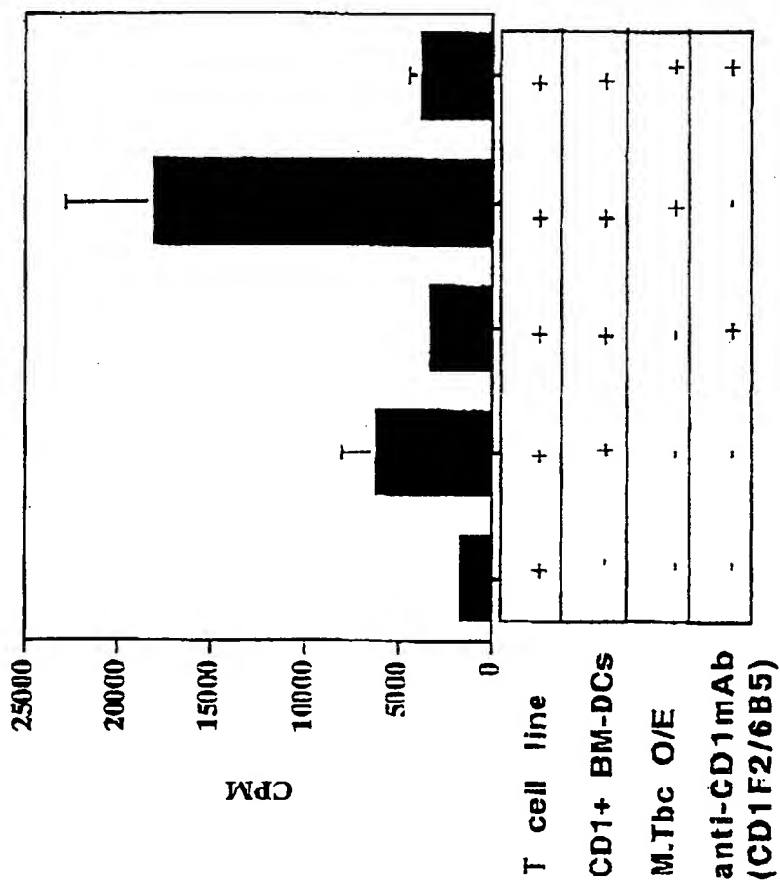
Figure 6

CD1b1-, CD1b2-, and CD1c3 restriction of cytolytic response of guinea pig S2-CD8.1 T cell lines



Fig. 7

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6   A61K39/04    A61K39/39    A61K39/00    A61K39/002   //(A61K39/39,
39:04),(A61K39/39,39:002),(A61K39/39,39:00)

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication. where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 96 12190 A (BRENNAN PATRICK J ;BRENNER MICHAEL B (US); BRIGHAM & WOMENS HOSPIT) 25 April 1996 (1996-04-25) | 1-3, 5-17, 19-24, 26-32, 34-41, 43-52,54 |
| | page 11, line 23 - page 12, line 13 page 36, line 1 - page 39, line 12 examples 10,12 claims 23,24,29-34 | |
| A | GONG J ET AL: "Isolation of mycobacterium -reactive CD1 -restricted T cells from patients with human immunodeficiency virus infection." JOURNAL OF CLINICAL INVESTIGATION, (1998 JAN 15) 101 (2) 383-9. , XP002109711 the whole document | 1-54 |

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[X] Further documents are listed in the continuation of box C.    [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 21 July 1999 | 03/08/1999 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Covone, M |

Form PCT/ISA/210 (second sheet) (July 1992)

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**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ' | Citation of document, with indication.where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | DORMAN SE ET AL.: "Adjunctive corticosteroid therapy for patients whose treatment for disseminated Mycobacterium avium complex infection has failed" CLINICAL INFECTIOUS DISEASES, vol. 26, no. 3, March 1998 (1998-03), pages 682-6, XP002109712 abstract see "Discussion" <br>--- | 1-54 |
| P,X, L | WO 99 12562 A (BRIGHAM & WOMENS HOSPITAL ;PORCELLI STEVEN A (US); MOODY D BRANCH) 18 March 1999 (1999-03-18) <br><br> page 3, line 13-15 <br> page 22, line 28 - page 23, line 8 <br> page 24, line 9 - page 30, line 7 <br> claims 19,20 <br>--- | 1-3, 5-17, 19-24, 26-32, 34-41, 43-52,54 |
| P,X | WO 98 39025 A (LENAERTS ANNE ;LEWIN JOHN HARVEY (GB); ADCOCK INGRAM LTD (ZA); JOH) 11 September 1998 (1998-09-11) <br><br> page 5, paragraph 6 <br> page 15, paragraphs 2-4 <br> claims 1-64 <br>--- | 1-3, 5-17, 19-24, 26-32, 34-41, 43-52,54 |
| P,X | GIULIANI A ET AL: "Effect of rifampin on CD1b expression and double-negative T cell responses against mycobacteria -derived glycolipid antigen." LIFE SCIENCES, (1998) 63 (12) 985-94. , XP002109713 abstract figure 2 see "discussion" <br>----- | 1,5-7, 26, 28-30,37 |

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# INTERNATIONAL SEARCH REPORT

| Box I | Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet) |
|---|---|

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:      1-25,37,47
   because they relate to subject matter not required to be searched by this Authority, namely:

   see FURHTER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

| Box II | Observations where unity of invention is lacking (Continuation of Item 2 of first sheet) |
|---|---|

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant. this International Search Report covers all
   searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee. this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant. this International Search Report
   covers only those claims for which fees were paid. specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

**Remark on Protest**      [ ] The additional search fees were accompanied by the applicant's protest.

     [ ] No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM    PCT/ISA/ 210**

Continuation of Box I.1

Although claims 1-25 37 47 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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Continuation of Box I.1

Claims Nos.:  1-25 37 47

Rule 39.1(iv)  PCT - Method for treatment of the human or animal body by therapy

| Inte  'ional Application No |
|---|
| PCT/US 99/08112 |

| Patent document cited in search report | | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|---|
| WO 9612190 | A | 25-04-1996 | US | 5853737 A | 29-12-1998 |
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| | | | AU | 4277996 A | 06-05-1996 |
| | | | CA | 2202680 A | 25-04-1996 |
| | | | EP | 0786088 A | 30-07-1997 |
| | | | JP | 10510142 T | 06-10-1998 |
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